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The feasibility of micro-ultrasound as a tool to image peripheral nerves

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Regional anaesthesia: mechanism of injury

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Summary

The incidence of transient and permanent nerve damage during regional anaesthesia has not changed despite widespread use of ultrasound imaging for needle guidance over the last decade. Current clinical ultrasound has insufficient anatomical resolution, and anaesthetists have difficulty judging the precise location of the needle tip relative to the epineurium. In view of the controversy surrounding intraneural injection, there is a need to understand the structural changes caused by subepineural and subperineural needle penetration. We present micro-ultrasound imaging, which offers anatomical resolution better than 100 μm , as a tool suitable for visualising neural anatomy and deformation caused by needle insertion. We imaged nine nerves from fresh and soft embalmed Thiel cadavers. Fascicles > 0.4 mm in width were identified. Subepineural needle placement was associated with denting, rotation and elastic deformation of fascicles, whereas subperineural needle insertion split fascicles permanently.

Introduction

Ultrasound guided regional anaesthesia (UGRA) has improved the efficacy of peripheral blocks, yet the incidence of nerve damage has not decreased over the last decade [1]. Nerve damage has been attributed to subperineural (intrafascicular) injection [2], but current clinical ultrasound systems have insufficient resolution to image nerves in detail, and anaesthetists have difficulty interpreting the anatomical relationships between the tip of the needle, connective tissue, epineurium, and intraneural nerve anatomy [3,4].

Even so, intraneural injection does not necessarily result in nerve damage in patients [5, 6]. Needle tips are more likely to lie within stromal tissue and local anaesthetic spreads between fascicles [7]. Recent animal studies, in contrast, have demonstrated nerve inflammation and haematoma after needle-nerve contact [8, 9] and decline in nerve electrophysiological function after non-specific intraneural injection [10].

No technology is currently available to clinicians that differentiates between subepineural and subperineural tissue during needle insertion. However micro-ultrasound, commonly used for pre-clinical imaging [11], has image resolution suitable for visualising neural anatomy. Micro-ultrasound uses transducer frequencies between 20 MHz and 100 MHz and offers resolution of anatomy between 150 μm and 30 μm respectively [11]. Micro-ultrasound using a single element transducer has been used to identify the epidural space [12], demonstrated in a needle to aid neurosurgical guidance to targets in fresh porcine and soft embalmed cadaver models [13], and, using a real-time imaging system, to visualise cutaneous nerves in the hand and wrist [18] .

The objective of this study was to assess the feasibility of micro-ultrasound imaging as a method to visualise fascicles within nerves resected from fresh and soft embalmed cadavers, and to observe any displacement or disruption of the neural anatomy caused by anaesthetic needle penetration. Our secondary objectives were to assess the integrity of fresh and Thiel soft embalmed nerves [17] on handling and during needle insertion, and compare micro-ultrasound imaging with histology sections.

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Methods

Ethical approval for the study using fresh and Thiel soft embalmed cadaveric tissue was obtained from the Thiel Advisory Committee, Centre for Human Anatomy and Identification (CAHID), University of Dundee. An anatomy scientific officer ensured that cadaver care and governance was undertaken according to the Anatomy Act, Scotland (2006). A senior anatomist dissected fresh median and femoral nerves from a recently deceased cadaver just prior to embalming (cadaver A) and median, radial, and femoral nerves from three soft embalmed Thiel cadavers (cadavers B, C and D). Specimen age, defined as the time since embalming, varied between 11 and 28 months, whereas fresh cadaver nerves were less than 48 hours old (Table 1). Nerves were dissected via the axillary and femoral incisions used for embalming in order to minimise tissue disruption.

Scanning

Dissected nerves were labelled and catalogued, then transported securely to the Institute of Science and Technology, University of Dundee at Ninewells Hospital. Specimens were placed in a petri dish partly filled with 3% agar, held in position using insect pins and bathed in deionised water for imaging (Fig 1). We scanned each nerve in six transverse, parallel planes, 0.5 mm apart, using focused single element 30 MHz and 45 MHz micro-ultrasound transducers (AFM Ltd, Birmingham, UK). The focal distance of each transducer is 5.6 mm and 4.3 mm respectively, and the lateral resolution is 0.14 mm and 0.11 mm respectively. Radiofrequency (RF) data acquired from the micro-ultrasound scanning system was converted into grey-scale B-mode images using engineering software (MATLAB, Natick, MA).

Needle insertion

For needle insertion tests, we designed a bespoke block using Solidworks (Waltham, MA) that attached to the petri dish and held a 22g regional block needle (B.Braun, Sheffield, UK) at a 45° angle (Fig 1). The needle block was manufactured in polylactide (PLA), a polymer, using a 3D printer (MakerBot, Brooklyn, NY). Needle insertion purposefully targeted subepineural or subperineural tissue. We scanned 3 median nerves at 3 distinct times: before needle insertion; with the needle inserted and after needle withdrawal. Ten or more transverse micro-ultrasound image slices, separated by 0.5 mm, were acquired around the insertion site and radiofrequency (RF) data processed in MATLAB (Natick, MA) data visualised as 2D and 3D images in ImageJ (NLM, Washington DC).

Histology

We performed histology on two nerves using a technique modified from standard histological staining to allow visualisation of myelin lipids [14]. Formaldehyde was used to fix proteins and 1% osmium tetroxide was used to fix and stain unsaturated lipids black in order to highlight the position of fascicles [15]. The fixed specimens were cut in transverse sections to a thickness between 8 µm and 10 µm using a microtome, and stained with haematoxylin and eosin (H&E) in order to differentiate nuclei and connective tissue.

In compliance with the Anatomy Act Scotland (2006) all specimens were returned to CAHID for cremation with the donor cadaver.

Statistical analysis

Two investigators identified fascicles in each ultrasound image and on histology. Nerve width was measured on ultrasound images and presented as mean (SD). No power analysis was performed as we were exploring the feasibility of micro-ultrasound imaging of peripheral nerves for the first time and had a limited number of specimens.

Results

Fifty four micro-ultrasound scans were performed on 9 nerves. The characteristics of each nerve are given in Table 1. In all scans we recognised epineurium as a discrete echogenic boundary, fascicles as round, slightly anechoic areas, and subepineural tissue as bright echogenic tissue between fascicles, but distinct from epineurium (Fig 2). Using micro-ultrasound all identifiable fascicles were >0.4 mm in diameter.

There was no difference in the quality of micro-ultrasound images obtained from resected from cadaver A (fresh, recently deceased), and cadaver B, which had been embalmed for 11 months. Nerves embalmed for 28 months, dissected from cadaver D, were fragile on handling, and disintegration of the epineurium was visible by naked eye and on micro-ultrasound images (e.g. Fig 2(d)).

Needle insertion was performed on three median nerves, with intrafascicular insertion targeted for the fresh nerve in cadaver A. Subepineural needle insertion into the embalmed median nerve of cadaver B required little manual force. Fig 3 (bottom row) shows fascicle rotation then return to pre-insertion shape with different orientation of the fascicles after needle removal. A slight increase in the width of the nerve specimen was noticed after the needle was withdrawn. This might be due to the rough handling of the nerves and also the excess pressure put on the perineurium to insert the needle into the fascicles. A 3D stack of images of the nerve with needle inserted is shown in a video (on-line Appendix).

Subperineural injection into fresh nerve was difficult. Fascicles had a tendency to rotate and 4 attempts were necessary to successfully insert needles through tough perineurium. The subperineural injection was confirmed through tactile feedback from the needle penetrating the perineurium and the observations during the experiments. Fig 3 (Upper row) shows a dent in the epineurium and splitting of the fascicle into two without return to normal shape, retaining the indentation.

Histology was conducted on both fresh and Thiel embalmed nerve specimens. The distribution and rank size of fascicles were similar to that observed with corresponding micro-ultrasound images.

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However, more fascicles were recognised using histology, and corresponded to those fascicles < 0.4 mm not seen on micro-ultrasound images. Of note, each specimen shrunk by approximately one third in size during histology because of the fixing process [15]. Histology of the fresh median nerve after needle insertion is shown in Fig 4 (upper row). Osmium tetroxide took time to diffuse deeper into the specimen, if the specimen used for histology is longer. The specimen was immersed in 1% Osmium tetroxide for 2 hours for it to diffuse into the nerve specimen. The specimens used for histology varied between 1 cm and 3 cm. Osmium tetroxide took less time to diffuse into the fresh cadaver nerve specimen as, the length of the nerve specimen used for histology was 1cm. Whereas, the Osmium tetroxide wasn't able to diffuse much into the Thiel nerve specimens as the length of the Thiel specimens used for histology was almost 3cm.

Discussion

Our study showed that micro-ultrasound imaging of peripheral nerves is feasible, and enabled us to differentiate for the first time between subepineural and subperineural needle insertion using micro-ultrasound images. We visualised rotation and elasticity of fascicles in response to needle tip pressure and mechanical trauma to fascicles in response to forceful needle insertion.

We conducted this study because intraneural injection is a contentious issue in regional anaesthesia, and deliberate or accidental intraneural injection in patients does not necessarily manifest as clinical neurological side effects [7]. Histology studies of intraneural injection have shown that needle tips are more likely to lie within stromal tissue [5,6], On the contrary, intraneural injection studies in animal models have shown a decrement in nerve electrophysiological function [10], and nerve inflammation and haematoma associated with forceful needle-nerve contact [8,9].

Our results indicate that micro-ultrasound supplements the use of histology and electrophysiology in intraneural injection experiments because it provides visible evidence of subepineural and subperineural morphology and trauma. We readily recognised fascicles as round, hypoechoic areas, and subepineural tissue as bright echogenic tissue residing between fascicles. Our results show fascicle

rotation secondary to subepineural needle insertion but we also observed that nerve fascicles are elastic and return to their original position after removal of the needle. Alarming, subepineural needle insertion traumatically split a fascicle into two, and segments failed to revert to pre-injection dimensions. We are unable to make any recommendation for practice based on this observation as only three needle insertions were performed, and only one was targeted for subepineural placement. We intend to conduct a follow-up study using a much larger number of cadaver nerves and inject fluid such as India ink into and around fascicles to track the needle tip position on histology slides.

Thiel soft embalmed cadavers provide tissue specimens with realistic anatomy and yield ultrasound images similar to clinical subjects [16]. Thiel soft embalmed cadavers retain the tissue elasticity and joint motion of live tissue or fresh cadaveric specimens, but are aseptic [17]. The University of Dundee has 80 soft embalmed cadavers that are used to teach anatomy to medical and dental students, train anaesthetists and surgeons, and provide a platform for medical technological testing, reducing the need for animal experimentation. For preserving the cadaveric tissues, tissue dissection and intravascular infusion of embalming solution is first performed in the axilla and groin, followed by soaking for 6 months in large metallic vats containing the embalming solution. The nerves used in this study were chosen as common target nerves for RA, and to minimise tissue disruption in the cadavers.

Both fresh nerves and Thiel soft embalmed nerves provided a satisfactory model for simulation of intraneural injection but still had limitations. Availability of fresh nerves was restricted, and experiments were conducted quickly before decomposition. By contrast, Thiel embalmed nerves felt softer than fresh nerve, and the epineurium of nerves from the cadavers embalmed 20 and 28 months tended to disintegrate with manual handling. Nevertheless, the pattern and distribution of fascicles >0.4 mm observed with micro-ultrasound imaging in these longer embalmed cadavers was maintained when compared to more recently embalmed specimens and fresh nerves. The deterioration observed in the 20 and 28 month old embalmed nerves was possibly due to dehydration

during storage in air, or ongoing embalming, causing continuing protein denaturation, after the embalming procedure is complete.

The quality of soft embalmed nerves at 11 months was similar to fresh nerves using micro-ultrasound imaging and in histology. Further research is required to understand changes in tissue microstructure over time. In the meantime, we would recommend that future studies be conducted on soft embalmed nerves less than 12 months old to avoid the tissue degeneration but take advantage of the long working time possible with Thiel embalmed tissues.

In conclusion, micro-ultrasound imaging visualised the anatomy of soft embalmed and fresh nerves differentiating clearly between subepineural and subperineural tissue. Subepineural injection was accompanied by rotation and elasticity of fascicles whereas subperineural needle insertion resulted in traumatic splitting of a fascicle.

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Table 1 Origin and measured characteristics of nerve specimens.

Cadaver	Type	Embalming age (Months)	Nerve	Side	Nerve width Mean (SD)	No. of visible fascicles	Needle insertion	Histology
A	Fresh	0	Femoral	Right	3.00 (0.12)	3		
			Median	Right	3.72 (0.12)	4	Yes	Yes
B	Thiel	11	Median	Right	4.90 (0.09)	6	Yes	
			Radial	Right	1.93 (0.12)	3		
			Femoral	Right	1.95 (0.14)	3		
C	Thiel	20	Median	Left	11.97 (0.14)	5		Yes
D	Thiel	28	Median	Left	7.00 (0.15)	3		
			Radial	Right	5.97 (0.15)	2		
			Median	Right	5.83 (0.12)	1	Yes	

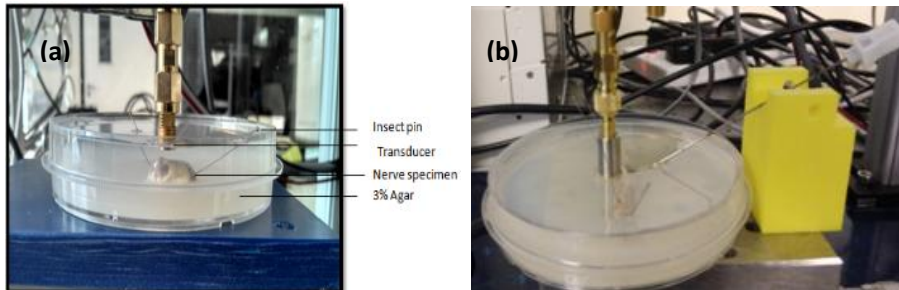


Fig 1. Micro-ultrasound imaging and nerve injection set-up. (a) The nerve specimen is placed in on 3% agar within an 88 mm petri dish and held in place with insect pins. The nerve and tip of the micro-ultrasound transducer are bathed in deionised water. (b) The needle support (yellow) holding a needle angled at 45° inserted into a nerve specimen.

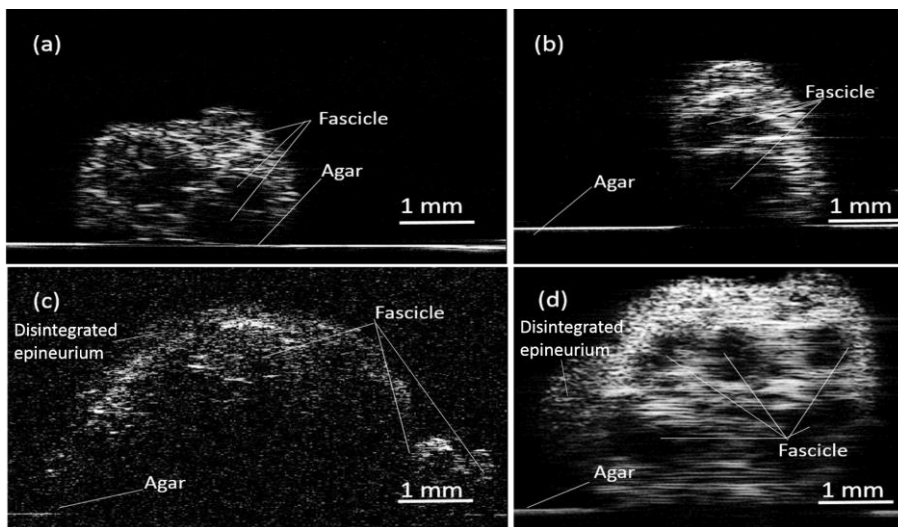


Fig 2. Representative micro-ultrasound scans of nerves resected from fresh and Thiel soft embalmed cadavers. (a) Fresh femoral nerve from Cadaver A; (b) radial nerve from Cadaver B, 11 months embalmed; (c) left median nerve from Cadaver C, 20 months embalmed; (d) median nerve from Cadaver D, 28 months embalmed. Agar substrate recognised as bright horizontal baseline. Fascicles

recognised as poorly echogenic round areas, surrounded by echogenic interfascicular tissue. Epineurium in (d) seen as fine honeycomb pattern extending from 9 o'clock to 1 o'clock, and indicative of epineural degeneration.

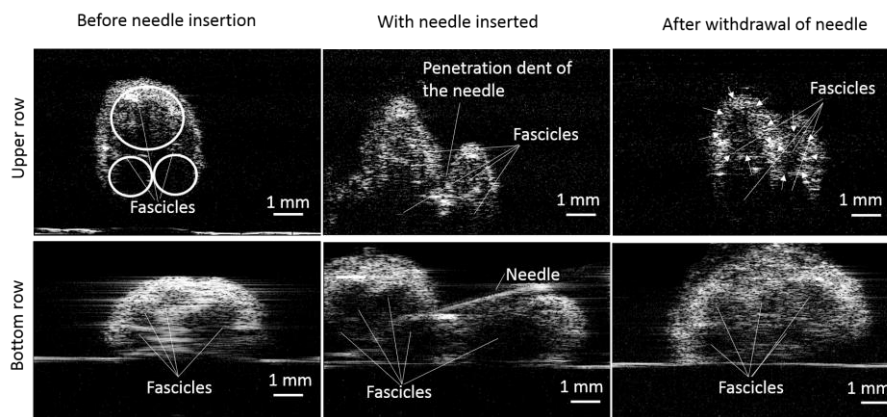


Fig 3. Micro-ultrasound images of needle insertion into nerve. Upper row shows fresh median nerve with fascicles and agar substrate identified. From left to right, images show nerve before needle insertion; needle denting and penetrating epineurium; splitting of nerve into two without return to pre-injection dimensions. Lower row shows nerve from from cadaver D, with fascicles identified. From left to right, images show nerve prior to needle insertion; needle denting epineurium; and tissue demonstrating elasticity with nerve returning to pre-insertion dimensions with different orientation of fascicles after needle removal

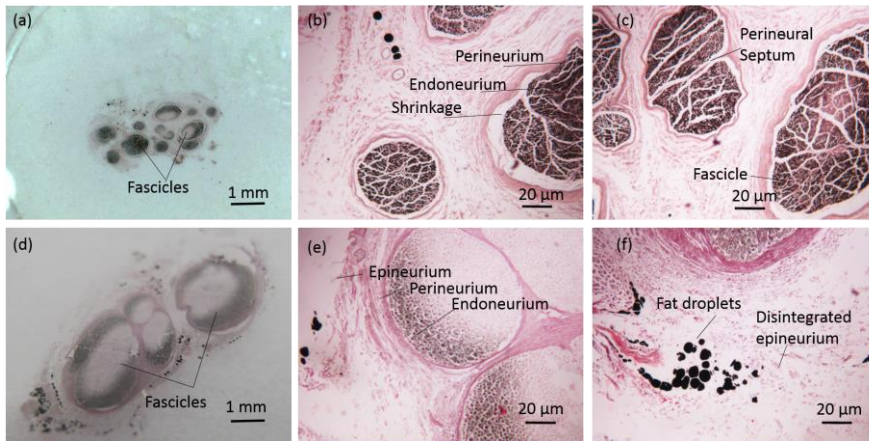


Fig 4. Histology images. Upper row shows fresh median nerve. Fascicle architecture more obvious in upper images. Linear strands within fascicles are indicative of differential shrinkage secondary to fixation and staining process. Lower row shows median nerve from 20 months embalmed Cadaver 3. Epineurium, perineurium and endoneurium are visible. Fat droplets indicative of tissue disintegration are highlighted by black droplets of osmium staining.

Appendix

3D Video of needle puncturing nerve